The specification has been objected to for failure to indicate the status of the application from which it claims priority.

The specification has been objected to for failure to comply with one or more of the requirements of 37 C.F.R. §§ 1.821-1.825 because the specification recites sequences that lack description by the appropriate sequence identifier set forth in the "Sequence Listing" as required by 37 C.F.R. § 1821(d).

Claims 1-12 and 15-23 are rejected under 35 U.S.C. § 112, first paragraph as lacking enablement commensurate with the scope of the claims.

Claims 1-14 and 22-25 are rejected under 35 U.S.C. § 112, second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 1-3, 6, and 10 are rejected under 35 U.S.C. § 102(b) as being anticipated by the Mizutani et al. journal article.

Claims 7-9 are rejected under 35 U.S.C. §103(a) as being unpatentable over the Mizutani et al. journal article.

Claims 4-5, 15-19, and 21 are rejected under 35 U.S.C. § 103(a) as being unpatentable over the Mizutani et al. journal article in view of the Davies et al. journal article.

Claim 20 is rejected under 35 U.S.C. § 103(a) as being unpatentable over the Mizutani et al. journal article in view of the Davies et al. journal article as applied to claims 4-5, 15-19, and 21, and further in view of the Vassal et al. journal article.

The formal drawings have been approved by the draftsperson.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached pages are captioned "Version with Markings to Show Changes Made". Material to be deleted is enclosed in brackets. Added language is underlined and bolded.

Claims 1 and 15 have been amended. Claims 10-14 and 21-25 have been canceled. No new matter has been added by virtue of the amendments to the claims and to the specification. Reconsideration of the application as amended and based on the arguments set forth below is respectfully requested.

II. Objections to the Specification

The specification has been objected to for failure to indicate the status of the application from which it claims priority. The specification has been amended to recite that the present application is a continuation-in-part of U.S. Application No. 09/323,472, filed June 1, 1999, now U.S. Patent No. 6,346,382.

The specification has been objected to for failure to comply with one or more of the requirements of 37 C.F.R. §§ 1.821-1.825 because the specification recites sequences that lack description by the appropriate sequence identifier set forth in the "Sequence Listing" as required by 37 C.F.R. § 1821(d). The specification has been amended to include sequence identifiers for those sequences that lacked them when the present U.S. patent application was originally filed. A substitute Sequence Listing

is enclosed herewith as both a computer-readable formatted (CRF) disk and a paper copy. The contents of the CRF of the substitute Sequence Listing are identical to the contents of the paper copy of the substitute Sequence Listing enclosed herewith. A statement to the effect is also enclosed. No new matter has been added. Thus, applicants believe this Response places the subject application into compliance with the requirements of 37 C.F.R. § 1.821-1.825. Applicants respectfully request that the CRF substitute Sequence Listing and paper substitute Sequence Listing be entered into the subject application.

III. Claim Rejections Under 35 U.S.C. § 112, First Paragraph

Claims 1-12 and 15-23 are rejected under 35 U.S.C. § 112, first paragraph, as lacking enablement commensurate with the scope of the claims. The United States Patent and Trademark Office (hereinafter the "Patent Office") acknowledges that the specification is "enabled for methods in which susceptibility to 'sub-optimal urea cycle function' and/or susceptibility to bone marrow transplant toxicity are determined in a human subject by detecting the presence of a threonine at amino acid 1405 of CPSI and by detecting a nucleotide sequence encoding that threonine in the CPSI gene". Official Action, page 2, paragraph 4. However, the Patent Office contends that the specification of the subject application does not reasonably provide enablement for "methods in which susceptibility to 'sub-optimal urea cycle function' and/or susceptibility to bone marrow transplant toxicity in any type of 'subject' are determined by detecting the presence of any polymorphism in the CPSI gene, including any C to A transversion in exon 36 of the CPSI gene." Official Action at page 3, paragraph 1. The specific arguments relied upon by the Patent Office are set forth in the Official Action at page 3, paragraph 2 - page 6, paragraph 1.

Claim 1 has been amended to recite a method of treating or preventing sub-optimal urea cycle function in a human subject having a polymorphism that results in a N→T substitution at amino acid 1405 of a carbamyl phosphate synthetase I (CPSI) polypeptide. Thus, amended claim 1 recites treating or preventing sub-optimal urea cycle function in a human subject who has a polymorphism that results in a threonine at amino acid 1405 of CPSI. As mentioned above, the Patent Office has acknowledged the specification is enabled as to this particular polymorphism. Applicants respectfully submit that amended claim 1 is believed to meet the enablement requirements of 35 U.S.C. § 112, first paragraph.

Claims 2-9 depend from amended claim 1, and thus include all amendments recited in claim 1. As such, claims 2-9 are believed to also comply with 35 U.S.C. § 112, first paragraph. Claims 10-12 have been canceled and this rejection of claims 10-12 under 35 U.S.C. § 112, first paragraph is thereby rendered moot. Thus, applicants respectfully request withdrawal of the rejection of claims 1-9 under 35 U.S.C. § 112, first paragraph.

Claim 15 has been amended to recite a method of treating or preventing bone marrow transplant toxicity in a human subject undergoing bone marrow transplant, the

human subject having at least one copy of a carbamyl phosphate synthetase I (CPSI) gene that encodes a threonine residue at amino acid 1405 of a CPSI polypeptide, the method comprising administering to the human subject a therapeutically effective amount of a nitric oxide precursor, whereby bone marrow transplant toxicity is treated or prevented in the human subject. Thus, amended claim 15 recites treating or preventing sub-optimal urea cycle function in a human subject whose genome contains at least one copy of the CPSI gene that encodes a threonine residue at position 1405 of a CPSI polypeptide as disclosed in the subject application, and is believed to meet the enablement requirements of 35 U.S.C. § 112, first paragraph. Support for this amendment can be found throughout the specification of the instant application, particularly on page 63, lines 4-5 and 11 (human subject), and page 87, lines 10-12 (threonine-encoding allele).

Claims 16-20 depend from amended claim 15, and thus include all the amendments recited in claim 15. As such, claims 16-20 are believed to also comply with 35 U.S.C. § 112, first paragraph. Claims 21-23 have been canceled and this rejection of claims 21-23 under 35 U.S.C. § 112, first paragraph is thereby rendered moot. Thus, applicants respectfully request withdrawal of the rejection of claims 15-20 under 35 U.S.C. § 112, first paragraph.

IV. Rejection of Claims under 35 U.S.C. § 112, Second Paragraph

Claims 1-14 and 22-25 are rejected under 35 U.S.C. § 112, second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

In the view of the Patent Office, claims 1-14 are indefinite based on the recitation of "subject in need thereof". Official Action, page 6, paragraph 4. The Patent Office suggests that the term "subject in need thereof" is unclear as to what subjects would be encompassed by this language. Official Action, page 6, paragraph 4. Claim 1 has been amended to remove this language, and claims 10-14 have been canceled. Thus, this rejection of claims 1-14 under 35 U.S.C. § 112, second paragraph is believed to be addressed.

In the view of the Patent Office, claims 11-14 and 22-25 are indefinite because "it is unclear as to how the further step of 'initially detecting a polymorphism' relates to treating or preventing sub-optimal urea cycle function as in claim 1 or to treating or preventing bone marrow transplant disease as in claim 15." Official Action, page 7, paragraph 1. Claim 11-14 and 22-25 have been canceled and this rejection of claims 11-14 and 22-25 under 35 U.S.C. § 112, second paragraph is thereby rendered moot.

In the view of the Patent Office, claims 12-14 and 23-25 are indefinite because the limitation "the polymorphism of the carbamyl phosphate synthetase polypeptide" lacks antecedent basis. Official Action, page 7, paragraph 2. Claims 12-14 and 23-25 have been canceled and this rejection of claims 12-14 and 23-25 under 35 U.S.C. § 112, second paragraph is thereby rendered moot.

The Patent Office contends that claims 13-14 and 23-24 are indefinite based on the language "cDNA that corresponds to the CPSI gene". Official Action, page 7, paragraph 3. Claims 13-14 and 23-24 have been canceled and this rejection of claims 13-14 and 23-24 under 35 U.S.C. § 112, second paragraph is thereby rendered moot.

V. *Rejection of Claims under 35 U.S.C. § 102(b)*

Claims 1-3, 6, and 10 have been rejected by the Patent Office under 35 U.S.C. § 102(b) as anticipated by the Mizutani et al. journal article (Tohuku L. Exp. Med. 142:15-24 [1984]; hereinafter "Mizutani"). The Patent Office contends that the treatment and prevention of hyperammonemia in humans with arginine and citrulline is disclosed. Official Action, page 8, paragraph 3. After careful consideration of the rejection, applicants respectfully traverse the rejection and submit the following comments.

It is well settled that for a cited reference to qualify as prior art under 35 U.S.C. §102, each element of the claimed invention must be disclosed within the reference. "It is axiomatic that for prior art to anticipate under 102 it has to meet every element of the claimed invention." Hybritech Inc. v. Monoclonal Antibodies, Inc., 802 F.2d 1367, 231 USPQ 81 (Fed. Cir. 1986). Upon careful consideration and review of Mizutani, applicants respectfully submit that the disclosure of Mizutani does not disclose each and every element of the present invention. Specifically, Mizutani does not disclose the polymorphism at amino acid 1405 of a carbamyl phosphate synthetase I (CPSI) polypeptide.

Claim 1 has been amended to recite a method of treating or preventing sub-optimal urea cycle function in a human subject having a polymorphism that results in a N→T substitution at amino acid 1405 of a carbamyl phosphate synthetase I (CPSI) polypeptide. The recited polymorphism is not disclosed in Mizutani. Claims 2-3 and 6 depend from amended claim 1 and thus are also believed to be patentably distinguishable from Mizutani. Claim 10 has been canceled and this rejection of claim 10 under 35 U.S.C. § 102(b) is thereby rendered moot. Applicants submit that, in view of the above amendment and remarks, Mizutani does not disclose each and every element of the present invention and applicants respectfully request that the rejection of claims 1-3 and 6 under 35 U.S.C. § 102(b) be withdrawn and the claims be allowed at this time.

VI. *Rejection of Claims under 35 U.S.C. § 103(a)*

Claims 7-9 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Mizutani. Official Action, page 9, paragraph 1. The Patent Office contends that Mizutani discloses the treatment of hyperammonemia in humans with arginine and citrulline, and further that arginine and citrulline were found to prevent hyperammonemia. The Patent Office further contends that Mizutani discloses that

dosages employed are determined by body weight and vary depending on the manner of administration. The Patent Office also contends:

> Given Mizutani et al's teachings regarding the varied response to arginine and citrulline in different patients, one of ordinary skill would have been motivated to have optimized dosages employed in different patients, and thereby to have employed lower dosages meeting the requirements of the claims when appropriate for a particular patient. Further, given Mizutani et al's teachings, one of ordinary skill would have been motivated to have employed smaller doses meeting the requirements of the claims in smaller patients (e.g., infants), for the advantage of providing patients of lower body weight with a dose of the correct concentration... Finally a skilled artisan would have been further motivated to have employed lower doses when administering arginine and/or citrulline intravenously, as Mizutani et al exemplify the use of intravenous concentrations lower than the oral concentrations employed.

Official Action, page 9, paragraph 2 – page 10, paragraph 1.

Claims 4-5, 15-19, and 21 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Mizutani in view of Davies et al. (Bone Marrow Transplantation 17:1119-1125 (1996); hereafter Davies). The Patent Office contends that Mizutani discloses that treatment with arginine and citrulline was found to prevent hyperammonemia, but does not disclose the treatment or prevention of "bone marrow transplant toxicity" in a subject undergoing bone marrow transplant. Official Action, page 10, paragraph 3. The Patent Office further contends that Davies discloses that hyperammonemia is a complication of BMT, and thus it would be *prima facie* obvious to one of ordinary skill to have modified the treatment of Mizutani to treat or prevent hyperammonemia in a bone marrow transplant patient. Official Action, page 10, paragraph 3 – page 11, paragraph 1. With respect to claims 17-19, the Patent Office contends that it would have been *prima facie* obvious to one of ordinary skill to have modified the method of Mizutani in view of Davies so as to have employed lower or optimized doses of arginine and/or citrulline either in smaller patients or when administered intravenously, thus meeting the requirements of the instant claims. Official Action, page 11, paragraph 2 – page 12, paragraph 1.

Claim 20 is rejected under 35 U.S.C. § 103(a) as being unpatentable over Mizutani in view of Davies as applied to claims 4-5, 15-19, and 21, above, and further in view of Vassal et al. (Cancer, Chemotherapy and Pharmacology 37:247-253 (1996); hereinafter Vassal). The Patent Office acknowledges that Mizutani and Davies "do not disclose administering arginine and/or citrulline to a patient suffering from hepatic veno-occlusive disease (HVOD)", but asserts further that, "Vassal et al disclose that HVOD, like hyperammonemia, is a complication of bone marrow transplantation." Official Action, page 12, paragraph 3. The Patent Office further contends that "[i]n view of the teachings of Vassal et al, it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Mizutani et al and Davies et al so as to have treated bone marrow transplant patients suffering from HVOD by administering arginine and/or citrulline." Official Action, page 12, paragraph 3 – page 13, paragraph 1.

After careful consideration of the rejections, applicants respectfully traverse the rejections and submit the following comments.

Preliminarily, applicants note that the U.S. Court of Appeals for the Federal Circuit (C.A.F.C.) has set forth in Environmental Design Ltd. v. Union Oil Co., 713 F.2d 693 (Fed. Cir. 1983), cert. denied, 464 U.S. 1043 (1984), that the factual determinations to be made, as well as the evidence to consider, in making an obviousness determination under §103 include:

a) the scope and content of the prior art;

b) the differences between the prior art and the claimed invention;

c) the level of ordinary skill in the pertinent art; and

d) additional evidence, which may serve as indicia of non-obviousness.

All relevant evidence on each of these four dispositive issues must be fully considered and evaluated to determine whether the claimed invention would have been obvious. Additionally, it is well known that for an obviousness-type rejection to stand, the cited document or combination must disclose all aspects of the claimed invention; contain a suggestion to modify the cited document(s) to arrive at the claimed invention; and there must be a reasonable chance of success.

In Hodosh v. Block Drug Co., 786 F.2d 1136 (Fed. Cir. 1986), the U.S. Court of Appeals for the Federal Circuit set forth what is described as the "tenets of patent law that must be adhered to when applying §103", Id. at 1143, n.5. Those tenets set out in Hodosh are:

a) the claimed invention must be considered as a whole;

b) the references must be considered as a whole and suggest the desirability and thus obviousness of making the combination;

c) the references must be reviewed without benefit of hindsight vision afforded by the claimed invention; and

d) "ought to be tried" is not the standard with which obviousness is determined.

Applicants respectfully submit that Mizutani does not meet the requirements for a *prima facie* case of obviousness, either alone, in combination with Davies, or in combination with Davies and Vassal.

The Patent Office has rejected claims 7-9 under 35 U.S.C. § 103(a) based on the disclosure of Mizutani. Claims 7-9 depend from amended claim 1. Claim 1 has been amended to recite a polymorphism that results in an N→T substitution at amino acid 1405 of a carbamyl phosphate synthetase I (CPSI) polypeptide, an element lacking from the cited reference. Additionally, Mizutani presents at best an "ought to be tried" scenario similar to that proscribed in Hodosh v. Block Drug Co. discussed above.

Claims 4-5, 15-19, and 21 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Mizutani in view of Davies. Claim 1 has been amended to recite a polymorphism that results in an N→T substitution at amino acid 1405 of a carbamyl

phosphate synthetase I (CPSI) polypeptide. Claims 4-5 depend from amended claim 1. As discussed above, Mizutani does not disclose this polymorphism, and applicants respectfully submit that Davies does not address this defect in the teachings of Mizutani. Applicants urge that the cited references, Mizutani and Davies, cannot be combined to either teach or suggest each and every element of the present invention and therefore that claims 4-5 are patentably distinguished over the cited references.

Similarly, claim 15 has been amended to recite a method of treating or preventing bone marrow transplant toxicity in a human subject undergoing bone marrow transplant, the human subject having at least one copy of a carbamyl phosphate synthetase I (CPSI) gene that encodes a threonine residue at amino acid 1405 of a CPSI polypeptide, the method comprising administering to the human subject a therapeutically effective amount of a nitric oxide precursor, whereby bone marrow transplant toxicity is treated or prevented in the human subject. Applicants urge that the cited references, Mizutani and Davies, cannot be combined to either teach or suggest the CPSI polymorphism that encodes a threonine residue at amino acid 1405 of the CPSI polypeptide. Thus, applicants respectfully submit that claims 15-19 and 21 are patentably distinguished over the cited references.

Claim 21 has been canceled and this rejection of claim 21 under 35 U.S.C. § 103(a) is thereby rendered moot. Applicants therefore respectfully request that the rejection of claims 4-5 and 15-19 under 35 U.S.C. §103(a) based on these documents be withdrawn and that the claims be allowed at this time.

In the alternative, even assuming arguendo that the combination of Mizutani with Davies discloses each and every element of the claimed invention as the Patent Office contends, applicants respectfully submit that the cited references offer no explicit or implicit suggestion to combine the cited references. Mizutani involves oral administration of arginine or citrulline as a treatment for Lysinuric Protein Intolerance (LPI). LPI is a disease caused by defective dibasic amino acid transport, not abnormal amino acid metabolism as might be experienced by subjects with CPSI defects. Thus, the etiology and treatment of the hyperammonemia suffered by LPI patients could be expected to differ significantly from those of patients suffering from CPSI defects. Davies, on the other hand, involves post-mortem studies of patients that had undergone BMT. In Davies, 12 patients were examined, 6 of whom had no discernible plasma amino acid or urea cycle defect, and 2 others who died despite treatment that returned ammonia levels to normal. These patients made up just 0.5% of the BMT patients present in the database (12 of 2358). As such, it is unlikely that one of ordinary skill in the art would perceive and/or consider arginine or citrulline treatment to be beneficial to BMT patients. Davies also suggested that hyperammonemia following BMT-related chemotherapy "is likely to occur in acutely ill patients...with no evidence of an underlying metabolic defect". Davies at page 1123. As such, applicants respectfully submit that one of ordinary skill in the art would not have been motivated to consider arginine or citrulline treatment to be beneficial to BMT patients.

Applicants respectfully submit that at best, the cited references are simply an "invitation to experiment" and present an "ought-to-be-tried" situation similar to the one discussed above. As the Federal Circuit stated in Hodosh v. Block Drug Co., "ought to be tried' is not the proper standard for determining obviousness. Applicants respectfully submit, therefore, that the cited references alone or in combination present an "ought-to-be-tried" situation and lack a suggestion to modify the references to arrive at the present invention with a reasonable expectation of success even if the references are combined as proposed by the Patent Office.

Claim 20 is rejected under 35 U.S.C. § 103(a) as being unpatentable over Mizutani in view of Davies as applied to claims 4-5, 15-19, and 21, above, and further in view of Vassal. Claim 20 depends from claim 15, which has been amended to recite a method of treating or preventing bone marrow transplant toxicity in a human subject undergoing bone marrow transplant, the human subject having at least one copy of a carbamyl phosphate synthetase I (CPSI) gene that encodes a threonine residue at amino acid 1405 of a CPSI polypeptide, the method comprising administering to the human subject a therapeutically effective amount of a nitric oxide precursor, whereby bone marrow transplant toxicity is treated or prevented in the human subject. Applicants urge that the cited references, Mizutani and Davies, whether or not in combination with Vassal, do not teach or suggest a human subject having at least one CPSI allele that encodes a CPSI polypeptide with a threonine at position 1405, and therefore that claim 20 is patentably distinguished over the cited references.

In the alternative, even assuming arguendo that the combination of Mizutani with Davies and Vassal discloses each and every element of the claimed invention as the Patent Office contends, applicants submit that the cited references offer no explicit or implicit suggestion to combine the cited references. As mentioned above, there is no suggestion to combine Mizutani with Davies. Applicants respectfully submit that the inclusion by the Patent Office of the Vassal reference does not provide this suggestion. There is no reference in Vassal to hyperammonemia, nor is there a suggestion that abnormal urea cycle function contributes to HVOD. Applicants respectfully submit that there is no suggestion presented in the references that BMT-related complications involve a common biochemical pathway, and thus no suggestion that a treatment strategy designed to impact one such pathway, namely the urea cycle, would be efficacious in treating these disparate complications. As such, applicants respectfully submit that one of ordinary skill in the art would not be motivated to treat BMT-related HVOD with a nitric acid precursor as recited in claim 20.

Applicants respectfully submit that at best, the cited references are simply an "invitation to experiment" and present an "out-to-be-tried" situation similar to the one discussed above. Applicants respectfully submit, therefore, that the cited references alone or in combination present an "out-to-be-tried" situation and lack a suggestion to

modify the references to arrive at the present invention with a reasonable expectation of success even if the references are combined as proposed by the Patent Office.

Summarily, applicants respectfully submit that the Patent Office has not presented a prima facie case of obviousness. As such, applicants further submit that dependent claims 4-5, 7-9, 15-19, and 20 are in condition for allowance and respectfully request that the rejection of claims 4-5, 7-9, 15-19, and 20 under U.S.C. §103(a) be withdrawn and that the claims be allowed at this time.

CONCLUSIONS

In light of the above amendments and remarks, applicants submit that the application is in condition for allowance and courteously solicit a Notice of Allowance.

If any small matter should remain outstanding after the Patent Examiner has had an opportunity to review the above Remarks, the Patent Examiner is respectfully requested to telephone the undersigned patent attorney in order to resolve these matters and avoid the issuance of another Official Action.

DEPOSIT ACCOUNT

The Commissioner is hereby authorized to charge any deficiencies of payment or credit any overpayments associated with the filing of this correspondence to Deposit Account No. 50-0426.

Respectfully submitted,
JENKINS & WILSON, P.A.

Date: January 3, 2003 By: Arles A. Taylor, Jr.

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1242/19/2 CIP AAT/CPP/ptw

Enclosures: Postcard
Sequence Listing in paper and computer readable form
Petition for Extension of Time
Amendment Transmittal

VERSION WITH MARKINGS TO SHOW CHANGES MADE

Deleted text is enclosed in brackets ([ ]). Text to be added is underlined and in bold typeface.

IN THE SPECIFICATION:

The paragraph beginning at page 1, line 5, has been amended as follows:

This is a continuation-in-part of U.S. Patent Application Serial No. 09/323,472, filed June 1, 1999, **now U.S. Patent No. 6,346,382,** the entire contents of which are herein incorporated by reference.

The paragraph beginning at page 7, line 1, has been amended as follows:

Preferably, the polymorphism of the carbamyl phosphate synthetase polypeptide comprises [a C to A] **an A to C** transversion in exon 36 of the CPSI gene, more preferably at nucleotide 4340 of a cDNA that corresponds to the CPSI gene. More preferably, the [C to A] **A to C** transversion at nucleotide 4340 of the cDNA that corresponds to the CPSI gene further comprises a change in the triplet code from AAC to ACC, which encodes a CPSI polypeptide having a threonine moiety at amino acid 1405.

The paragraph beginning at page 19, line 11, has been amended as follows:

The primers of the invention embrace oligonucleotides of sufficient length and appropriate sequence so as to provide initiation of polymerization on a significant number of nucleic acids in the polymorphic locus. The CPSI locus is depicted schematically in Fig. 5. Specifically, the term "primer" as used herein refers to a sequence comprising two or more deoxyribonucleotides or ribonucleotides, preferably more than three, and more preferably more than eight and most preferably at least about 20 nucleotides of the CPSI gene wherein the DNA sequence contains the [C to A] **A to C** transversion at base 4340 relative to CPSI contained in SEQ ID NO's:1 and 3. The allele including cytosine (C) at base 4340 relative to CPSI is referred to herein as the "CPSIa allele", the "T1405 allele", or the "threonine-encoding allele". The allele including adenosine (A) at base 4340 relative to CPSI is referred to herein as the "CPSIb allele", the "N1405 allele", or the "[arginine] **asparagine**-encoding allele".

The paragraph beginning at page 56, line 17, has been amended as follows:

Where a CPSI gene itself is employed it will be most convenient to simply use a wild type CPSI gene directly. The CPSI gene can thus comprise the threonine encoding allele such that amino acid 1405 of the encoded polypeptide comprises threonine. Alternatively, the CPSI gene comprises the [arginine] **asparagine** encoding allele such that amino acid 1405 of the encoded polypeptide comprises [arginine] **asparagine**. Additionally, it is envisioned that certain regions of a CPSI gene can be employed exclusively without employing an entire wild type CPSI gene or an entire

allelic variant thereof. It is proposed that it will ultimately be preferable to employ the smallest region needed to modulate the urea cycle so that one is not introducing unnecessary DNA into cells which receive a CPSI gene construct. Techniques well known to those of skill in the art, such as the use of restriction enzymes, will allow for the generation of small regions of an exemplary CPSI gene. The ability of these regions to modulate the urea cycle can easily be determined by the assays reported in the Examples. In general, techniques for assessing the modulation of the urea cycle are known in the art.

The paragraph beginning at page 61, line 23, has been amended as follows:

Optionally, the supplementation therapy method of the present invention further comprises the step of initially detecting a polymorphism of a carbamyl phosphate synthase I (CPSI) gene in the subject. The polymorphism of the carbamyl phosphate synthetase polypeptide preferably comprises [a C to A] **an A to C** transversion within CPSI exon 36, more preferably comprises [a C to A] **an A to C** transversion at nucleotide 4340 of a cDNA that corresponds to the CPSI gene, and [ever] **even** more preferably, the [C to A] **A to C** transversion at nucleotide 4340 of the cDNA that corresponds to the CPSI gene further comprises a change in the triplet code from AAC to ACC, which encodes a CPSI polypeptide having a threonine moiety at amino acid 1405.

The paragraph beginning at page 83, line 16, has been amended as follows:

Genotyping. DNA was isolated using a QIAmp™ blood kit (Qiagen). The T1405N polymorphism changes the DNA sequence as follows:

CCT-GCC-ACC-CCA-GTG **(SEQ ID NO:21)** Normal

CCT-GCC-AAC-CCA-GTG **(SEQ ID NO:22)** Change

The paragraph beginning at page 84, line 21, has been amended as follows:

In accordance with the present invention, a common polymorphism near the 3' end of the CPSI mRNA (about .44 heterozygosity) has been identified. Sequence analysis of this change revealed a C to A transversion at base 4340 changing the triplet code from ACC to AAC. This results in a substitution of asparagine for threonine at amino acid 1405 (referred to herein as "T1405N"). The threonine is within the allosteric domain, preceding the signature sequence PV(A/S)WP(T/S)(A/Q)E **(SEQ ID NO:23)**, a sequence that is important in the binding of the cofactor n-acetyl-glutamate (NAG).

The paragraph beginning at page 98, line 9, has been amended as follows:

Since there is no gender disparity in the occurrence of HVOD, we concentrated on potential pharmacogenetic issues related to CPSI, an autosomally encoded gene, rather than on the X-linked ornithine transcarbamylase gene. While characterizing the molecular changes underlying the causes of neonatal and late-onset CPSI deficiency,

a common SNP near the 3' end of the CPSI mRNA (0.44 heterozygosity) was identified. This C4340A transversion encodes a predicted substitution of asparagine (AAC) for threonine (ACC) at amino acid 1405 (T1405N). This threonine is within the allosteric domain, preceding the sequence PV(A/S)WP(T/S)(A/Q)E **(SEQ ID NO:23)** important in the binding of a cofactor, n-acetyl-glutamate (NAG), that increases enzyme activity. Although applicants do not wish to be bound by any particular theory of operation, it is speculated that based on the precedent of the effects of other xenobiotics, that limited availability of NAG after escalated dose chemotherapy is one of the mechanisms promoting urea cycle dysfunction. Nonetheless, it appears that the presence of the CPS-I SNP AA genotype is associated with protection against the development of HVOD, resolution of ALI if it occurs, and improved 60 day survival after BMT. Thus, the data suggest that alteration in UC function plays a role in modifying liver-lung interaction during sepsis and acute lung injury.

The paragraph beginning at page 115, line 10, has been amended as follows:

The T1405N allele exhibits 50% heterozygosity and appears to be a silent variant in normal healthy adults. However, consequences of the qualitative change can be unmasked by stressful conditions. As disclosed in Examples 1-3, **studies on** adults exposed to high-dose chemotherapy in preparation for bone marrow transplantation **demonstrated** that the threonine-containing enzyme produces inadequate levels of arginine and citrulline and is associated with an increased incidence of hepatic veno-occlusive disease, acute lung injury, and death. As nitric oxide (NO) is generated in endothelial cells from L-arginine by nitric oxide synthetase (NOS), decreased levels of urea cycle intermediates could predispose to disturbances in vascular tone by limiting endogenous NO production.

Please delete the pending sequence listing and insert in place thereof the sequence listing attached hereto.

IN THE CLAIMS:

Please cancel claims 10-14 and 21-25.

Claim 1 has been amended as follows:

1. (Once Amended) A method of treating or preventing sub-optimal urea cycle function in a **human** subject **having a polymorphism that results in a N→T substitution at amino acid 1405 of a carbamyl phosphate synthetase I (CPSI) polypeptide**, the method comprising administering to [a] **the human** subject [in need thereof] a therapeutically effective amount of a nitric oxide precursor, whereby treatment or prevention of sub-optimal urea cycle function is accomplished.

Claim 15 has been amended as follows:

15. (Once Amended) A method of treating or preventing bone marrow transplant toxicity in a **human** subject undergoing bone marrow transplant, **the human**

subject having at least one copy of a carbamyl phosphate synthetase I (CPSI) gene that encodes a threonine residue at amino acid 1405 of a CPSI polypeptide, the method comprising administering to the **human** subject a therapeutically effective amount of a nitric oxide precursor, whereby bone marrow transplant toxicity is treated or prevented in the **human** subject.